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The subsensitivity of striatal glutamate receptors induced by chronic haloperidol in rats

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Abstract

The aim of the present study was to investigate the influence of chronic treatment with haloperidol on the contralateral head turns and rotations induced by intrastriatal agonists of NMDA and non-NMDA receptors in rats. *N*-Methyl-D-aspartate (NMDA, 500 ng/0.5 μl), α-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA, 1000 ng/ 0.5 μl) or kainic acid (50 ng/0.5 μl), injected into the intermediate and caudal parts of the caudate-putamen, induced contralateral head turns and rotations. Haloperidol was given to animals in a dose of ca. 1 mg/kg per day in drinking water for 6 weeks. On day 5 of withdrawal, haloperidol decreased the number of contralateral head turns, but did not significantly influence the contralateral rotations induced by NMDA, AMPA and kainic acid. At the same time, haloperidol enhanced the stereotypy induced by apomorphine (0.25 mg/kg s.c.). The present results seem to suggest that, apart from supersensitivity to dopamine, chronic treatment with haloperidol also induces subsensitivity of striatal NMDA and non-NMDA receptors.

Keywords: Haloperidol; NMDA receptor; Non-NMDA receptor; Subsensitivity; Striatum; Contralateral turning

1. Introduction

It is well known that chronic treatment with antipsychotic drugs induces tardive dyskinesia in humans. It has been postulated that this disorder results from the supersensitivity of striatal dopamine D_2 receptors, developed as compensation for the prolonged blockade of these receptors by a neuroleptic (for references see Wolfarth and Ossowska, 1989). However, the pathophysiology of tardive dyskinesia seems to be more complex, and the dopaminergic supersensitivity theory has been questioned (for references see Wolfarth and Ossowska, 1989). It has also been found that neuroleptics, via the blockade of dopamine receptors, influence GABAergic striatal output pathways which convey impulses to the globus pallidus, substantia nigra and entopeduncular nucleus as well (Mao et al., 1977; Itoh, 1983; Gunne and Häggström, 1983; Gunne et al., 1984; Johnson et al., 1994). Changes in the GABAergic transmission in the above structures have been suggested to contribute to the neuroleptic-induced extrapyramidal side-effects, including tardive dyskinesia (Gunne and Häggström, 1983; Fibiger and Lloyd, 1984; Gunne et al., 1984; Ossowska et al., 1984; Ellenbroek et al., 1985; Scheel-Krüger, 1986; Johansson and Gunne, 1989; Ossowska et al., 1993).

The GABAergic output striatal pathways seem to be under the stimulatory influence of a glutamatergic corticostriatal projection (Gerfen, 1992). In the striatum, a considerable number of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors are co-localized with dopamine ones on GABAergic striatal output neurons (Joyce and Marshall, 1987; Greenamyre and Young, 1989; Smith and Bolam, 1990; Gerfen, 1992; Tallaksen-Greene et al., 1992). A disturbed balance between dopaminergic and glutamatergic transmission in the striatum has been suggested to be responsible for symptoms of Parkinson's disease, as well as for drug-induced parkinsonism – an acute side-effect of a treatment with neuroleptics (Klockgether and Turski, 1989; Carlsson and Carlsson, 1990; Riederer et al., 1991, 1992; for references see Ossowska, 1994).

To test the hypothesis that the interaction between dopaminergic and glutamatergic receptors may be important for not only the acute but also the chronic effects of neuroleptics, we examined the influence of

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chronic treatment with the classic neuroleptic haloperidol on striatal glutamate receptors in rats. We found recently that stimulation of striatal NMDA, α-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA) and kainate receptors by unilateral injection of their specific agonists into the ventral part of the intermediate-caudal striatum induced contralateral turning (Ossowska and Wolfarth, 1994, 1995). In the present study we used that effect as a behavioral index of sensitivity of striatal NMDA and non-NMDA receptors.

2. Materials and methods

The experiment was performed on male Wistar rats. Haloperidol base (RBI) was dissolved in a small amount of lactic acid and was then diluted with tap water. Haloperidol was given in a dose of ca. 1 mg/kg per day in drinking water for 6 weeks to 44 animals, and was afterwards withdrawn. To monitor the intake of the appropriate dose of haloperidol, rats were weighed twice a week. The solution consumption was checked every day and the volume of the haloperidol solution was corrected the following day. 43 control animals received tap water ad libitum.

2.1. Apomorphine-induced stereotypy

The experiment was carried out on non-operated rats. Apomorphine hydrochloride (Sandoz) was injected in a dose of 0.25 mg/kg s.c. to rats treated chronically with haloperidol ($n = 10$) on day 5 of withdrawal, and to control animals ($n = 10$). The rats were rated for the presence and severity of apomorphine-induced stereotypy between 15–30 min after the injection, using a modified scale of Rupniak et al. (1984) where: 0 = no stereotypy, 1 = sniffing episodes or discontinuous sniffing, 2 = continuous sniffing, 3 = continuous sniffing and occasional licking, gnawing or biting, 4 = discontinuous sniffing and discontinuous licking, gnawing or biting, 5 = continuous licking, gnawing or biting. A particular behavior was scored as continuous when it was uninterrupted for at least a few minutes.

2.2. Intrastriatal injections

34 rats treated with haloperidol and 33 controls were implanted unilaterally with stainless steel guide cannulas (0.4 mm o.d.) under pentobarbital anaesthesia (Vetbutal, Biowet, Poland). The operation was performed 2–3 days before the end of haloperidol administration. Approximately 1 week after the surgery (on day 5 of haloperidol withdrawal), the animals were injected with drugs via an inner cannula (0.3 mm o.d.) that protruded 0.6 mm from a guide cannula. The tips of the inner cannulas were directed at the ventrolateral part of the intermediate and caudal regions of the caudate-putamen (A = 7020–6570; L = 3.4; H = −0.8 to −1.8) according to the Stereotaxic Atlas of König

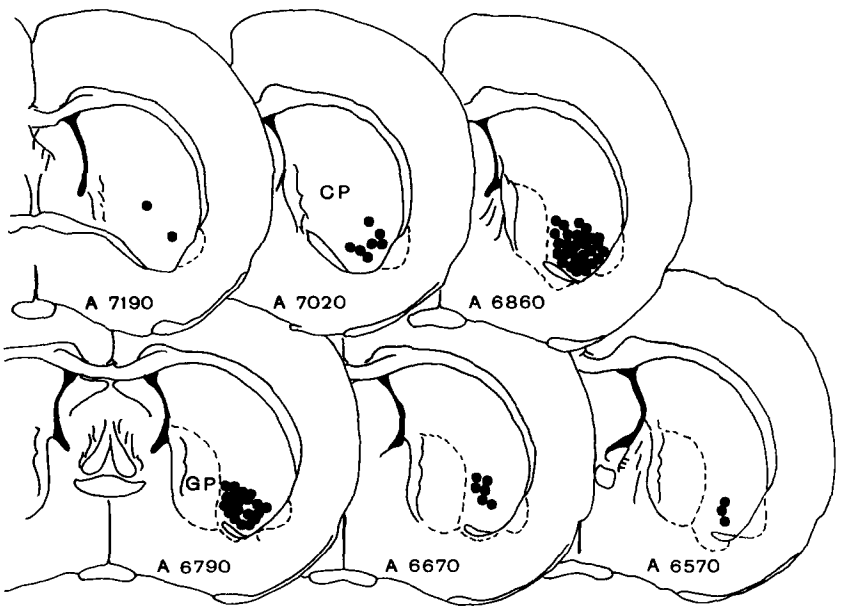

Fig. 1. Localization of cannula tips on frontal sections of the rat brain. Filled circles – cannula tips located in the ventrolateral part of the intermediate and caudal regions of the caudate-putamen (CP). Each circle denotes cannula placement in one animal. A – anterior plane, according to König and Klippel (1963), GP – globus pallidus.

and Klippel (1963) (Fig. 1). Injection of a volume of 0.5 μl lasted 2.0 min, and the inner cannula was withdrawn 1 min after the termination of the experiment.

N-Methyl-D-aspartate (NMDA, RBI, 500 ng) and α-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA, RBI, 1000 ng) were dissolved in small amounts of 1 N NaOH, diluted with redistilled water, and the pH was balanced with 1 N HCl to a final value of 6–7. Kainic acid (Sigma, 50 ng) was dissolved in distilled water. Intrastriatal NMDA injections were given to seven rats treated chronically with haloperidol, and to ten control animals. AMPA was injected into 13 haloperidol-treated and 10 control rats, and kainic acid into 14 haloperidol-treated and 13 control animals. Each animal was given one intrastriatal injection only.

2.3. Turning behavior

The number of contralateral and ipsilateral rotations and head turns was estimated by an observer during three 10-min periods. Head turns, i.e. ipsi- or contralateral head movements, were counted when an animal did not make any locomotor movements. Rotations were estimated as the turning of the whole body by 360° round the vertical axis.

After completion of the experiment, all the rats were killed by an overdose of pentobarbital, their brains were removed, and localization of all the injection cannula tips was checked histologically.

2.4. Statistics

For the turning behavior, a statistical evaluation of differences between groups was carried out by Student's *t*-test for independent variables, and for the differences between contralateral and ipsilateral turns – by Student's *t*-test for dependent variables.

For the stereotyped behavior, the Mann-Whitney U-test was used.

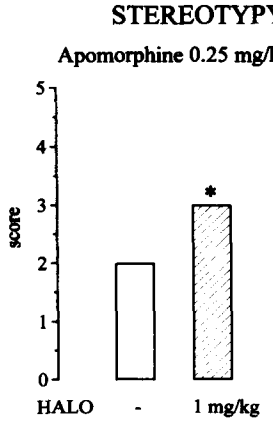

Fig. 2. The influence of chronic haloperidol (HALO) administration (1 mg/kg per day p.o.) on the stereotypy induced by apomorphine. Control animals received tap water instead of haloperidol. The results are presented as median values; the number of animals: control rats – $n = 10$; HALO – $n = 10$; an asterisk denotes a statistically significant difference at $P < 0.05$ vs. control rats.

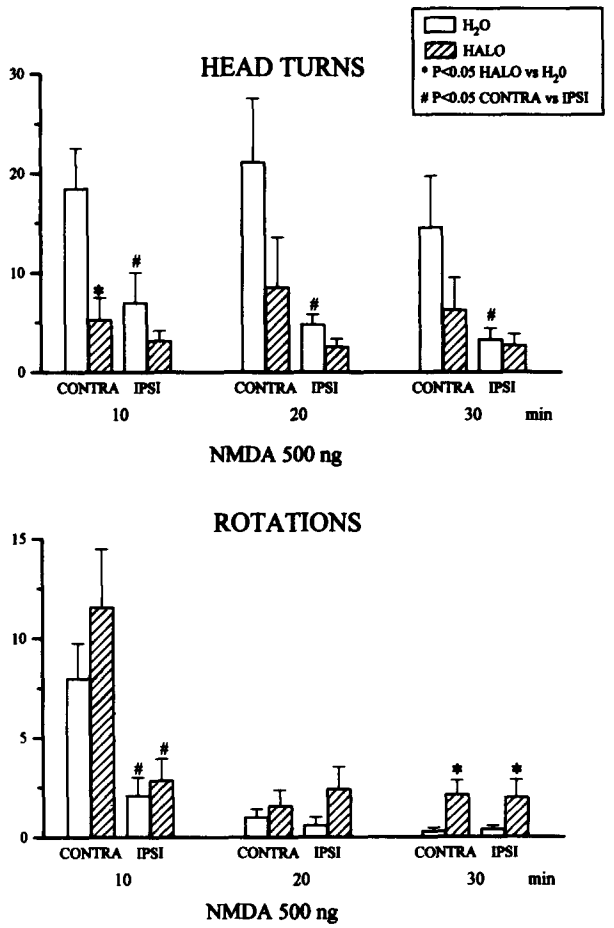

Fig. 3. The influence of chronic haloperidol (HALO) administration (1 mg/kg per day p.o.) on the contralateral head turns and rotations induced by unilateral intrastriatal NMDA injections. Control animals received tap water (H_2O) instead of haloperidol. Ordinate – the number of contralateral (CONTRA) or ipsilateral (IPSI) head turns or rotations; the results are presented as the means ± S.E.M.; the number of animals: H_2O + NMDA – $n = 10$; HALO + NMDA – $n = 7$; * statistically significant difference at $P < 0.05$ between haloperidol-treated and control rats; # statistically significant difference at $P < 0.05$ between the number of contra- and ipsilateral head turns or rotations within a group of haloperidol-treated or control animals.

3. Results

3.1. The influence of chronic haloperidol on the apomorphine-induced stereotypy

A 6-week treatment of rats with haloperidol (1 mg/kg per day p.o.) significantly enhanced the stereotypy induced by apomorphine (0.25 mg/kg s.c.), as

measured on day 5 of withdrawal (Fig. 2). In control rats, only discontinuous or continuous sniffing was observed. In contrast, the majority of haloperidol-treated rats showed licking episodes, discontinuous or continuous licking.

3.2. The influence of chronic haloperidol on the turning behavior induced by agonists of NMDA and non-NMDA receptors

NMDA (500 ng/0.5 μl), injected unilaterally into the ventrolateral part of the intermediate and caudal regions of the caudate-putamen, induced contralateral turning. The number of contralateral head turns and rotations differed significantly from the number of ipsilateral ones. That behavior was weak and relatively short-lasting. It consisted mainly of contralateral head turns, but few contralateral rotations were also observed (Fig. 3). Chronic treatment with haloperidol decreased the number of contralateral head turns, but did not visibly influence contralateral rotations (Fig. 3). At 30 min after NMDA injection to the haloperidol-treated group, a simultaneous small but statistically significant, increase in the number of contralateral and ipsilateral rotations was observed, which seemed to be related to the slight enhancement of the locomotor activity only.

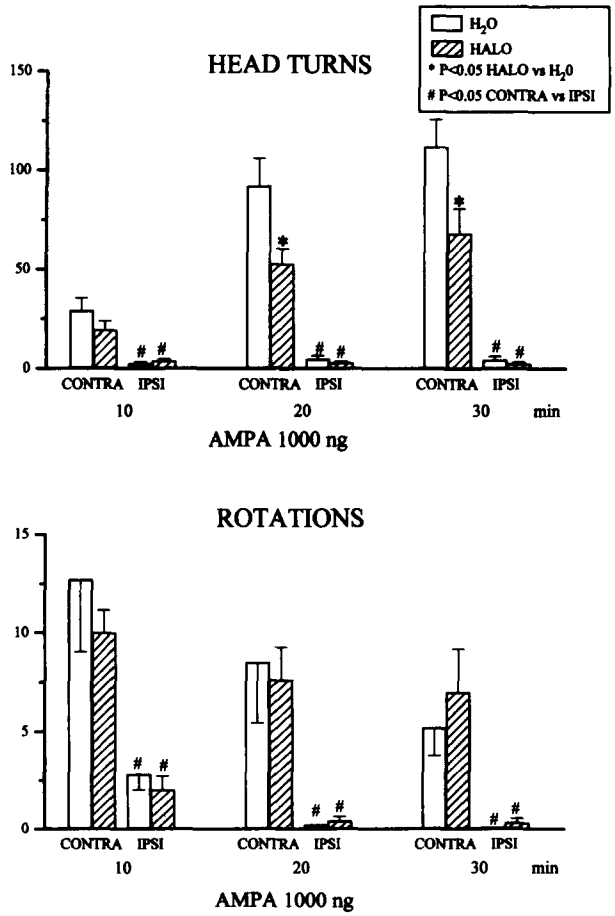

Fig. 4. The influence of chronic haloperidol (HALO) administration (1 mg/kg per day p.o.) on the contralateral head turns and rotations induced by unilateral intrastriatal AMPA injections. H_2O + AMPA – $n = 10$; HALO + AMPA – $n = 13$. For further explanations see Fig. 3.

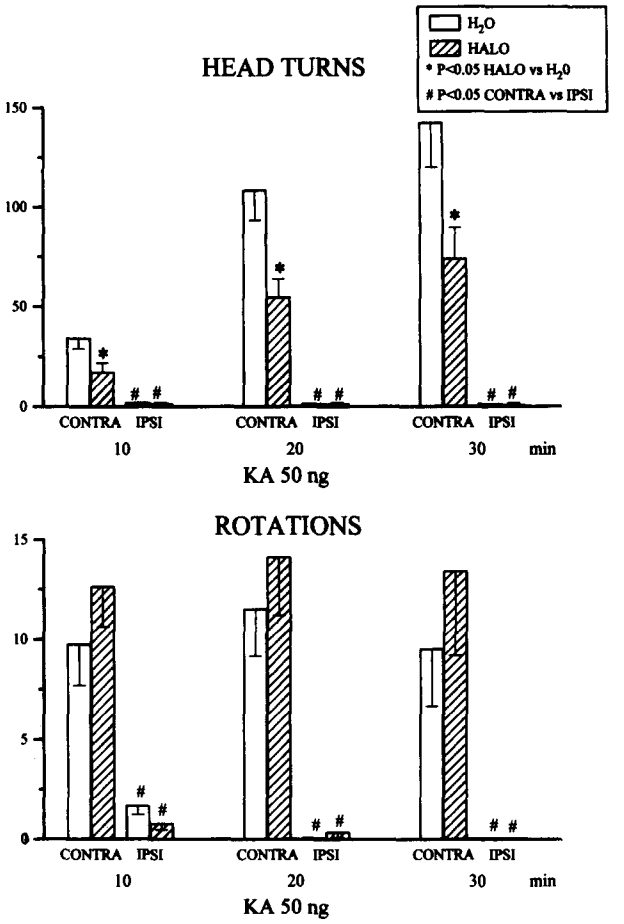

Fig. 5. The influence of chronic haloperidol (HALO) administration (1 mg/kg per day p.o.) on the contralateral head turns and rotations induced by unilateral intrastriatal kainic acid (KA) injections. H_2O + KA – $n = 13$; HALO + KA – $n = 14$. For further explanations see Fig. 3.

AMPA (1000 ng/0.5 μl) and kainic acid (50 ng/0.5 μl), injected unilaterally into the above-mentioned striatal regions, induced strong and long-lasting contralateral turning (Figs. 4 and 5). The number of contralateral head turns and rotations differed significantly from the number of ipsilateral ones. Like the behavior evoked by NMDA, the latter also consisted mainly of contralateral head turns. Chronic treatment with haloperidol decreased the number of contralateral head turns, but not the number of contralateral rotations induced by AMPA and kainic acid (Figs. 4 and 5).

4. Discussion

The present study shows that chronic, 6-week treatment with haloperidol decreases the number of contralateral head turns induced by injections of NMDA, AMPA and kainic acid into the ventrolateral parts of the intermediate and caudal regions of the caudate-putamen. That effect was observed on day 5 of the withdrawal when the whole amount of the neuroleptic was washed out from the brain tissue, which was proved by an increase in the apomorphine-induced stereotypy.

Our recent studies indicated that contralateral asymmetry induced by the above-mentioned compounds could be a good behavioral marker of the sensitivity of NMDA and non-NMDA receptors in that striatal region (Ossowska and Wolfarth, 1994, 1995). Therefore the present results suggest that chronic treatment with haloperidol leads to subsensitivity of NMDA and non-NMDA receptors localized in the ventrolateral parts of the intermediate and caudal regions of the caudate-putamen.

The finding that such a treatment with haloperidol increases the apomorphine-induced stereotypy corroborates numerous earlier studies which suggested that chronic blockade of dopamine receptors by the neuroleptic resulted in supersensitivity to dopamine (for references see Wolfarth and Ossowska, 1989). The present study also indicates that the increase in sensitivity of striatal dopamine receptors parallels in time the decrease in sensitivity of striatal NMDA and non-NMDA receptors.

Up to the present, there have been no other data available indicating the subsensitivity of striatal NMDA and non-NMDA receptors to be a result of chronic treatment with neuroleptics. A recent autoradiographic study (Johnson et al., 1994) did not show any changes in the binding of [^{3}H]AMPA to AMPA receptors, or in the binding of [^{3}H]MK-801 to phencyclidine sites of the NMDA receptor complex in the caudate-putamen after 6-month of administration of another classic neuroleptic, fluphenazine decanoate. Such a discrepancy between the above-mentioned study and our results may be due to the different neuroleptics used, as well as from diverse ways and time of their administration. Moreover, a change in binding sites is only one of the factors responsible for the sensitivity of receptors. A number of other processes may also contribute to the subsensitivity of striatal glutamate receptors to the behaviorally measured action of their agonists.

It is not quite clear on which populations of the striatal neurons these subsensitive NMDA and non-NMDA receptors are localized. We have recently suggested that contralateral head turns induced by NMDA, AMPA and kainate injections into the above-mentioned striatal region may result from stimulation of the strionigral pathway, and from subsequent enhancement of the GABAergic transmission in the substantia nigra pars reticulata (Ossowska and Wolfarth, 1994, 1995). The above conclusion is in line with some recent binding studies which show that part of striatal NMDA and non-NMDA receptors are present on strionigral neurons (Tallaksen-Greene et al., 1992). Some other anatomical as well as behavioral data support the latter suggestion. It has been shown that in the intermediate and caudal parts of the caudate-putamen there are a number of GABAergic strionigral neurons (Grofová, 1975; Araki et al., 1985; Beckstead and Cruz, 1986). In contrast, neurons of the striopallidal pathway (leading to the external segment of the globus pallidus) have been reported to be sparse in the caudal region of the striatum (Beckstead and Cruz, 1986). Moreover, contralateral turning occurs also after injections of GABA receptor agonists and substance P or dynorphin (peptidides co-localized with GABA in the strionigral pathway) into the substantia nigra pars reticulata (Wolfarth et al., 1981; Coward, 1982; Scheel-Krüger, 1986; for references see Reiner and Anderson, 1990). Although it cannot be excluded that our injections also affected some neurons of the GABAergic striopallidal pathway, such an effect does not seem to be involved in contralateral head turns, since unilateral enhancement of the GABAergic transmission in the globus pallidus has been reported to induce an opposite effect, i.e. ipsilateral turning (Scheel-Krüger, 1986). In conclusion, it is assumed that NMDA, AMPA and kainate receptors localized on the strionigral pathway are rendered subsenstitive as a result of chronic treatment with haloperidol.

In the present study haloperidol diminished contralateral head turns but not contralateral rotations induced by agonists of NMDA and non-NMDA receptors. It seems that contralateral head turns and rotations are mediated by different subpopulations of striatal neurons, which are equipped with glutamate receptors, but only some of them are influenced by haloperidol.

The direct cause of developement of subsensitivity of striatal excitatory amino acid receptors after haloperidol (which blocks predominantly dopamine D_2 receptors) is still obscure. It has been postulated that dopamine D_2 receptors in the caudate-putamen are localized mainly on the GABAergic striopallidal pathway (Gerfen, 1992). Therefore it is assumed that a prolonged blockade of these receptors by haloperidol increases secondarily, via a long (strio-pallido-nigro-thalamo-cortico-striatal) neuronal loop, striatal glutamatergic transmission (Carlsson and Carlsson, 1990) which, in turn, leads to a compensatory subsensitivity of postsynaptic glutamate receptors. The above assumption is based on the well-known fact that the cortico-striatal projection that closes the above-mentioned loop is glutamatergic (Carlsson and Carlsson,

1990, Gerfen, 1992). Moreover, this concept seems to be supported by the results which showed that agonists of dopamine D_2 receptors decreased, while chronic haloperidol increased the level and release of glutamate in the striatum (Maura et al., 1988; Yamamoto and Davy, 1992; Moghaddam and Bunney, 1993; See and Chapman, 1994).

Summing up, the obtained results suggest that chronic blockade of dopamine receptors by the neuroleptic leads not only to the well-known supersensitivity to dopamine, but also to subsensitivity of NMDA and non-NMDA receptors. The importance of the latter effect in the development of neuroleptic side-effects in humans, e.g. tardive dyskinesia, still remains to be assessed.

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